Arch. Environ. Contam. Toxicol. 44, 467–475 (2003)
DOI: 10.1007/s00244-002-2127-x

ARCHIVES OF
Environmental
Contamination
and Toxicology


# Toxicokinetics of Organic Contaminants in *Hyalella azteca*

Susanna Nuutinen,[1,2] Peter F. Landrum,[4] Lance J. Schuler,[3] Jussi V. K. Kukkonen,[2,4] Michael J. Lydy[1,3]

[1] Department of Biological Sciences, Wichita State University, 1845 Fairmount, Wichita, Kansas 67260-0026, USA
[2] Laboratory of Aquatic Ecology and Ecotoxicology, Department of Biology, University of Joensuu, P.O. Box 111, FIN-80101, Joensuu, Finland
[3] Fisheries and Illinois Aquaculture Center and Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901-6501, USA
[4] Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, 2205 Commonwealth Boulevard, Ann Arbor, Michigan 48105, USA

Received: 13 June 2002/Accepted: 21 October 2002

**Abstract.** Uptake, biotransformation, and elimination rates were determined for pentachlorophenol (PCP), methyl parathion (MP), fluoranthene (FU), and 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP) using juvenile *Hyalella azteca* under water-only exposures. A two-compartment model that included biotransformation described the kinetics for each chemical. The uptake clearance coefficients ($k_u$) were 25.7 ± 2.9, 11.5 ± 1.1, 184.4 ± 9.3, and 251.7 ± 9.0 (ml $g^{-1}$ $h^{-1}$) for PCP, MP, FU, and HCBP, respectively. The elimination rate constant of the parent compound ($k_{ep}$) for MP was almost an order of magnitude faster (0.403 ± 0.070 $h^{-1}$) than for PCP and FU (0.061 ± 0.034 and 0.040 ± 0.008 $h^{-1}$). The elimination rate constants for FU and PCP metabolites ($k_{em}$) were similar to the parent compound elimination 0.040 ± 0.005 $h^{-1}$ and 0.076 ± 0.012 $h^{-1}$, respectively. For MP, the metabolites were excreted much more slowly than the parent compound (0.021 ± 0.001 $h^{-1}$). For PCP, FU, and MP whose metabolites were measured, the biological half-life ($t_{1/2p}$) of the parent compound was shorter than the half-life for metabolites ($t_{1/2m}$) because the rate is driven both by elimination and biotransformation processes. Thus, *H. azteca* is capable of metabolizing compounds with varying chemical structures and modes of toxic action, which may complicate interpretation of toxicity and bioaccumulation results. This finding improves our understanding of *H. azteca* as a test organism, because most biomonitoring activities do not account for biotransformation and some metabolites can contribute significantly to the noted toxicity.

Developing and improving new models and approaches to assess the impacts of contaminants in the aquatic environment requires improved knowledge of fundamental toxicological processes. Toxicological processes can be divided into three general phases; 1) exposure, 2) toxicokinetics, and 3) toxicodynamics (McCarty and Mackay 1993). The exposure phase includes the period of time an organism is exposed to a chemical and the relative bioavailability of the chemical during that exposure. The toxicokinetic phase includes the uptake, distribution, metabolism, and elimination of the bioavailable portion of the toxicant. The toxicodynamic phase involves the biological response resulting from the chemical arriving at the site(s) of toxic action in the organism and acting to produce its toxic effect(s) in a time dependant manner (McCarty and Mackay 1993). Although our general understanding of these processes is improving, there remains a paucity of information for many important aquatic organisms, including those selected as standard test organisms, such as *Hyalella azteca*.

*Hyalella azteca* (Amphipoda: Hyalellidae) occurs widely throughout North and Central America. As a result of its wide distribution, ease of culture, and sensitivity to contaminants, *H. azteca* was selected as one of three species for use in standardized toxicity test procedures for contaminated freshwater sediments (USEPA 2000; ASTM 2000). In addition, this organism has been used in many toxicity and bioaccumulation assays (Kane Driscoll *et al.* 1997a, b; McNulty *et al.* 1999; Blockwell *et al.* 1999; Duan *et al.* 2000; Ingersoll *et al.* 2000; Schuler *et al.* 2002). However, the fundamental disposition of contaminants within *H. azteca* has not been well studied. While *H. azteca* is capable of metabolizing environmental contaminants such as anthracene (Landrum and Scavia 1983), fluoranthene (Kane Driscoll 1997b), and DDT (Lofuto *et al.* 2000) there are limited data on biotransformation rates (Landrum and Scavia 1983) and toxicokinetics (Landrum and Scavia 1983; Lotufo *et al.* 2000; Steevens and Benson 2001). Therefore, it is important to determine *H. azteca's* ability to metabolize different types of chemicals, exhibiting different modes of toxic action and presumably different metabolic pathways. This information will allow for a better understanding of how *H. azteca* processes chemicals improving interpretation of bioassay results. Further, as the use of body residue as a dose metric is explored and expanded (e.g., McCarty and Mackay 1993), understanding and interpreting the body residue in light of the toxicokinetics and metabolic ability of organisms will permit better interpretation of the hazard represented by such residues.

Therefore, this paper focuses on the toxicokinetic modeling of several organic contaminants in *H. azteca.* The design for this study provides improved estimates for biotransformation

*Correspondence to:* M. J. Lydy; *email:* mlydy@siu.edu

and elimination processes. The objectives were: (1) to determine the toxicokinetic parameters of four model compounds, pentachlorophenol (PCP), methyl parathion (MP), fluoranthene (FU), and 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP), that are expected to be processed by different metabolic pathways; and, (2) to expand the application of improved toxicokinetic experimental design and models developed by Lydy *et al.* (2000).

## Materials and Methods

### *Organisms*

*Hyalella azteca* were obtained from existing cultures in the Environmental Toxicology Core Facility in the Department of Biological Sciences at Wichita State University. *H. azteca* were maintained in mixed-age cultures according to standard operating procedures of the USEPA for static cultures (USEPA 2000). Juvenile *H. azteca* that passed through a 1-mm sieve, but were retained on a 500-mm sieve (2–3 weeks old) were used in the experiments.

### *Chemicals*

The $^{14}$C-radiolabelled chemicals used in this study included pentachlorophenol (>98% purity, 11.9 mCi/mmol), methyl parathion (>94% purity, 13.8 mCi/mmol), fluoranthene (>99% purity, 52.0 mCi/mmol), and 2,2′, 4,4′, 5,5′-hexachlorobiphenyl (>95% purity, 12.6 mCi/mmol). Pentachlorophenol, MP, and HCBP were purchased from Sigma Chemical (St. Louis, MO), while FU was purchased from ChemSyn Laboratories (Lenexa, KS). Chemical purity of the radiolabelled compounds was confirmed using a Hewlett Packard series 1100 high-pressure liquid chromatograph (HPLC) and/or thin layer chromatography (TLC). Details concerning the separations are described in the biotransformation section. Non-radiolabelled compounds were purchased from Chem Service Co. (West Chester, PA).

### *Uptake Experiments*

Uptake experiments were performed under static conditions in 600-ml beakers. One day before the start of the exposure, a bulk aliquot (25 L) of reconstituted Moderately Hard Water (MHW, USEPA 2000) was spiked with individual test chemicals in an acetone carrier. The acetone level never exceeded 100 μl per liter of water and most likely evaporated during the one-day holding period. In the case of PCP and FU, an isotopic dilution of $^{14}$C-radiolabelled plus non-labeled compounds was used to achieve the appropriate concentration for the test. Methyl parathion and HCBP exposures were run with the radiolabelled compound only, since an isotopic dilution would have reduced the radioactivity level below that desirable for measurement. Nominal water concentrations were 132.3 μg/L PCP, 2.3 μg/L MP, 45.1 μg/L FU, and 3.0 μg/L HCBP. These concentrations were selected based on preparation for a larger project that will investigate the critical body residue for these compounds in *H. azteca.* In general, the concentrations were set at $LC_1$ levels with the exception of HCBP that is not soluble enough to result in toxicity in aqueous solutions. Spiked water was placed in the refrigerator (4°C) in the dark prior to use. The following day, 400 ml of spiked water was poured into each of the 28 exposure beakers and 40 animals were gently placed into each of the beakers. One-ml water samples were taken from each exposure beaker, placed into 10 ml of ScintiSafe 50% scintillation cocktail (Fisher Scientific, Fair Lawn, NJ) and quantified with liquid scintillation counting (LSC, Packard 1900 TR Liquid Scintillation Analyzer, Meridian, CT). Beakers were then covered with aluminum foil to reduce evaporation and placed into a Precision Scientific environmental chamber (Chicago, IL) at 22°C and with a 16 light:8 dark photoperiod. The light source for all of the experiments was yellow light ($\lambda > 500$ nm), and was used to minimize possible photodegradation of the compounds. Finally, water parameters of temperature, pH, dissolved oxygen, and conductivity were recorded at the beginning and end of each experiment.

Animals were not fed during the experiments, since feeding might complicate estimation of the toxicokinetic parameters. Both animal and water samples were taken at 0, 2, 4, 6, 8, 12, 16, and 24 h. Four replicate beakers were sampled at each sampling time. Animals were filtered with vacuum from the exposure water, rinsed with distilled water, and blotted dry. *H. azteca* from each replicate were then separated into two groups. The first 10 animals were weighed (to the nearest 1.0 μg on a Cahn C-33 microbalance Cahn Instruments INC, Cerritos, CA), placed directly into 10 ml of scintillation cocktail and sonicated for one minute on pulse mode using a Tekmar Model #TM501 Sonic Disruptor (Cincinnati, OH). These animals were used as a measure of total radioactivity. The remaining animals from the four replicates were pooled, weighed, placed into 5 ml of acetonitrile (ACN) and frozen at −20°C. These animals were used to measure metabolite formation. The metabolites were determined as detailed in the biotransformation section.

All scintillation counting was performed for 10 min per vial using automatic quench control. Sample counts were corrected for background and quench using the external standards ratio method.

### *Depuration Experiments*

The contaminant loading for the depuration study was conducted in 1-L beakers with 100 *H. azteca* placed into each beaker. The water-animal ratio was the same as in the uptake experiments, with one animal per 10 ml of exposure water. After a 24-h exposure period, animals were gently sieved from the dosed water, and rinsed with MHW. This collection time was considered time zero for the depuration experiment. Depuration experiments were conducted in 40 250-ml beakers with 30 *H. azteca* in each beaker. Beakers were covered with 300 μm mesh netting (Argent Chemical Laboratories, Redmond, WA) to prevent escape of test animals from the beakers, while they were immersed in a 20-L aquarium. Aquaria contained 17-L of MHW and a constant flow-through system, at the flow rate of 20-L a day, to keep radioactivity in the water at background levels during the depuration experiments. Both animal and water samples were taken at 0, 2, 4, 8, 12, 16, 24, 36, 48, and 72 h. Four replicate beakers were sampled at each sampling time. Sampling and analysis were performed using the same methods as described in the uptake section.

### *Biotransformation*

Frozen *H. azteca* samples were thawed, sonicated in acetonitrile, and centrifuged at 3,000 g for 15 min. The supernatant was then transferred to an evaporation vial and the pellet washed with 3-ml of methanol, vortexed, and centrifuged. The washing step was repeated and the methanol supernatants combined with the acetonitrile supernatant. The extract was then concentrated to 500 μl under a stream of nitrogen. Radioactivity of the remaining pellet was measured and these counts represented unextractable tissue bound metabolites. The concentrated extracts were then introduced into either an HPLC (FU. MP) or spotted onto a thin layer chromatography (TLC) plate (PCP). HPLC/TLC was used to fractionate the extract into parent compound and polar metabolites. Fractions were quantified with LSC in order to determine the ratio of parent and metabolites. The mass balance of radioactivity for FU, PCP, MP, and HCBP was measured as (*H. azteca* extract + *H.*

*azteca* residue)/total, averaged 86 ± 22%, 86 ± 25%, 111 ± 11%, and 112 ± 10% (mean ± SD, $n$ = 17), respectively.

Separation of parent compound and metabolites for MP and FU was accomplished using a Hewlett Packard 1100 HPLC attached to a Foxy Junior Fraction Collector (Isco, Lincoln, NE). Detection of compounds was accomplished using an UV VIS detector set at 254 nm. Fractions containing parent compound were collected 0.5 min before and after the retention time of the non-radiolabeled parent compound standard. Metabolite standards were not available; however, all fractions collected before the parent fractions were considered to be polar metabolites. All fractions were analyzed for $^{14}C$ activity to quantify the amount of parent and metabolite. The analytical column was a 25-cm C18 Hybersil (Hewlett Packard). Acetonitrile (ACN) and water were used as the mobile phase. The gradient elution for MP started at 30% ACN, increased to 70% ACN during the first five minutes, and from 70 to 100% during the last five minutes of the 10 min run. Fluoranthene was run isocratically using a 50:50 water and acetonitrile mixture for 10 min.

For PCP, the separation of parent compound and metabolites was performed by thin layer chromatography (TLC). TLC analyses were conducted on silica gel coated glass plates with a UV indicator using a hexane:acetone (60:40 vol/vol) solvent system. The plates were spotted with both concentrated extract and PCP standard. After development, the PCP standard was visualized and area corresponding to the standard was marked for the extracts. The plate was then subdivided into six increments from the origin to the location of the parent compound. The silica gel was scraped from the plate and placed in scintillation cocktail for LSC analysis. The increments corresponding to the parent compound were combined and all other increments were assumed to be metabolite.

## *Data Analysis*

The uptake and elimination of the chemicals were determined using a two-compartment model to describe the distribution of compounds in *H. azteca* (Lydy *et al.* 2000) (Fig.1). During the uptake phase, parent compound reversibly entered compartment $C_p$ from the water at a rate characterized by $k_u$. Chemical may leave the $C_p$ compartment either by elimination ($k_{ep}$) or by biotransformation ($k_m$) to compartment $C_m$. The rate constant $k_{em}$ describes the elimination of metabolites from $C_m$.

The data were collected so that the uptake and elimination phases could be modeled simultaneously. The data were fit by performing an iterative least squares fit to the following differential equations (1–3) using the fourth-order Runga-Kutta approach in the software package, Scientist, version 2.01 (MicroMath, Salt Lake City, UT).

$$\frac{dC_{tot}}{dt} = (k_u C_w) - (k_{ep} C_p) - (k_{em} C_m) \quad (1)$$

$$\frac{dC_p}{dt} = (k_u C_w) - (k_m C_p) - (k_{ep} C_p) \quad (2)$$

$$\frac{dC_m}{dt} = (k_m C_p) - (k_{em} C_m) \quad (3)$$

Where: $C_{tot}$ is the concentration of total compound in the animal (ng $g^{-1}$), $C_p$ is the concentration of parent chemical in the animal (ng $g^{-1}$), $C_m$ is the concentration of metabolites in the animal (ng $g^{-1}$), $C_w$ is the concentration of the chemical in water (ng $ml^{-1}$), $k_u$ is the uptake clearance coefficient (ml $g^{-1}$ $h^{-1}$), $k_{ep}$ is the parent compound elimination rate constant ($h^{-1}$), $k_m$ is the metabolite formation constant ($h^{-1}$), $k_{em}$ is the metabolite elimination rate constant ($h^{-1}$), and t is the time (h).

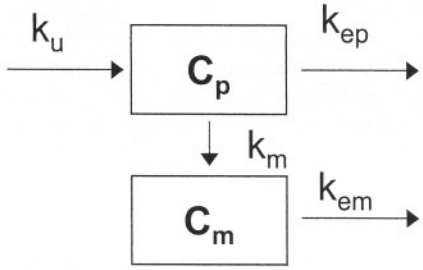


**Fig. 1.** Two compartment toxicokinetic model for chemicals in *Hyalella azteca* ($C_p$ = concentration of parent compound in the animal; $C_m$ = concentration of metabolite in the animal; $k_u$ = uptake clearance coefficient; $k_{ep}$ = parent compound elimination rate constant; $k_m$ = metabolite formation constant; $k_{em}$ metabolite elimination rate constant)

To use the model, estimates of the four initial parameters ($k_u$, $k_{em}$, $k_{ep}$, and $k_m$) were needed. To estimate $k_u$, the uptake data for the total radioactivity were fit to a two-compartment model (Landrum *et al.* 1992).

$$\frac{dC_{tot}}{dt} = (k_u \cdot C_w) - (k_d \cdot C_{tot}) \quad (4)$$

Where $k_d$ is the total compound elimination rate constant ($h^{-1}$).

To obtain estimates for $k_{ep}$, $k_{em}$, and $k_m$, we initially fit the depuration data to the appropriate one-compartment model (Equations 5–7). An additional parameter $k_{dp}$ was included as a measure of the disappearance of parent compound from the organism.

$$\frac{dC_{tot}}{dt} = -(k_d \cdot C_{tot}) \quad (5)$$

$$\frac{dC_p}{dt} = -(k_{dp} \cdot C_p) \quad (6)$$

$$\frac{dC_m}{dt} = -(k_{em} \cdot C_m) \quad (7)$$

Where $k_{dp}$ is the disappearance of parent compound ($h^{-1}$).

Based on the $k_d$ and $k_{dp}$ estimates, the initial parameters $k_{ep}$ and $k_m$ were calculated (Equations 8 and 9).

$$k_{ep} = k_d - k_{em} \quad (8)$$

$$k_m = k_{dp} - k_{ep} \quad (9)$$

Some modifications for the model were required for modeling MP and FU toxicokinetics. For example, to properly estimate all four parameters for MP, $k_u$ had to be held to a constant level of 11.5 ml $g^{-1}$ $h^{-1}$, which was set based on the initial modeling of the total radiolabeled compound uptake (Equation 4). This was necessary, since the $k_u$ estimate from the four-parameter fit was very poor (SD was 46% of the estimate). Constant infusion kinetic models generally assume that water concentrations will remain constant (<10% change) throughout the uptake portion of the experiment. However, when fitting by numerical integration, this is not a requirement and the data can be fit so long

as the relationship between the change in water concentration and time can be represented accurately. In the case of FU, the water concentration decreased 20% during the uptake phase of the experiment. This decline in chemical concentration in the water was taken into account by adding equation 10 to the overall model.

$$C_w = (0.42 \pm 0.02) - (t \cdot 48.6 \pm 0.2) \quad (10)$$

Bioconcentration factors (BCF) for the parent compound were estimated from the kinetics using the following equation:

$$BCF = \frac{C_p}{C_w} - \frac{k_u}{(k_{ep} + k_m)} \quad (11)$$

To properly evaluate a BCF for a metabolized compound, both the elimination rate of the parent compound ($k_{ep}$) and the loss rate via biotransformation ($k_m$) must be considered.

The biological half-lives of the parent compound ($t_{1/2p}$) and metabolites ($t_{1/2m}$) were determined by using the following equations:

$$t_{1/2p} = \frac{0.693}{(k_{ep} + k_m)} \quad (12)$$

$$t_{1/2m} = \frac{0.693}{k_{em}} \quad (13)$$

Linear regression analysis was used to measure the change in water concentration during the uptake phase, and to determine whether the weight of the test animals changed during the exposures. A significance level of 0.05 was employed to detect statistical difference. Analyses were performed with SPSS for Windows, release 10.1.0 (SPSS Inc. Chicago, IL).

## Results

### *Modeling*

Many factors can influence the observed toxicokinetics of aquatic organisms including environmental parameters such as temperature and physiological parameters such as organism size. To make comparisons among the compounds, the exposure conditions were controlled. Exposure water temperature was maintained at 21 ± 1°C, conductivity remained between 335–350 µS/cm, pH ranged from 7.8 to 7.9 and dissolved oxygen from 7.6 to 7.8 mg/L. A small decline in water concentration was observed, for PCP, MP, and HCBP; however, the decline was less than 5%, therefore it was not incorporated into the modeling process. The FU water concentration declined 20% during the uptake phase ($F = 370$, $df = 1,54$, $p < 0.05$). This decline was corrected by adding Equation 10 to the overall model.

Simple toxicokinetic models also assume that the weight of exposed organisms does not change during the experiment. Further, to compare among studies, the size of the organisms must be the same, since size can greatly affect $k_u$ (Landrum 1988; Landrum *et al.* 2001). The mean weights of the *H. azteca* did not change during the 96-h tests and the sizes of the organisms were similar among tests. Measured mean weights per amphipod during the 96-h test were: 0.67 ± 0.20 mg. 0.68 ± 0.14 mg, 0.65 ± 0.12 mg, and 0.66 ± 0.15 mg (mean ± SD, $n = 130$) for PCP, MP, FU, and HCBP tests, respectively.

A two-compartment model (Lydy *et al.* 2000) that partitioned the organisms into two homogenous compartments, one for the parent compound and a second for the metabolites was used to describe the kinetics of PCP, MP, and FU (Fig.1). The coefficients of determination (COD) for the overall models were good to excellent, 0.76, 0.90, and 0.83 for PCP, MP, and FU, respectively (Table 1). A simpler one-compartment model was used to determine the kinetics for HCBP because of the absence of biotransformation. The COD for the fitted equation for HCBP was high (0.83) for this simpler model (Table 1).

### *Pentachlorophenol*

The toxicokinetics of PCP are greatly affected by the pH at which the experiment is conducted due to ionization of the compound. The pKa for pentachlorophenol is 4.74 (Westhall 1985). Thus, at the pH used in the current studies (e.g. 7.8–7.9), PCP was largely ionized. The resulting uptake clearance ($k_u$) by *H. azteca* was 25.7 ± 2.9 ml · g$^{-1}$ h$^{-1}$ (Fig. 2). The elimination rate constants for parent compound and metabolites ($k_{ep}$ and $k_{em}$) (0.061 ± 0.034 and 0.076 ± 0.012 h$^{-1}$) were similar suggesting that there was equal resistance to elimination likely due to the ionized state of the compounds. The PCP biotransformation rate ($k_m = 0.133 \pm 0.017$ h$^{-1}$) was slower than that estimated for MP, but faster than for FU (see below). The BCF calculated from the kinetics for PCP was 132. The biological half-life of the parent compound ($t_{1/2p}$) was 3.6 h, and half-life of metabolites ($t_{1/2m}$) was 9.1 h (Table 1).

### *Methyl Parathion*

The uptake clearance ($k_u$) of MP by *H. azteca* was slow at 11.5 ± 1.1 ml g$^{-1}$ h$^{-1}$ (Fig. 2) reflecting the relatively small log $K_{ow}$ (2.04) for this compound, while the elimination of the parent compound ($k_{ep} = 0.403 \pm 0.070$ h$^{-1}$) was relatively fast. On the other hand, MP metabolites were eliminated very slowly ($k_{em} = 0.021 \pm 0.001$ h$^{-1}$). The biotransformation rate for MP ($k_m = 0.539 \pm 0.095$ h$^{-1}$) was rapid compared to the rate for the other compounds. The BCF for MP calculated from the kinetics was 12. The biological half-life of the parent compound ($t_{1/2p}$) was 0.7 h, and half-life of metabolites ($t_{1/2m}$) was 33.1 h (Table 1).

### *Fluoranthene*

The uptake clearance ($k_u$) of FU by *H. azteca* was 184.4 ± 9.3 ml g$^{-1}$ h$^{-1}$ (Fig. 2), while the elimination rate constants for parent compound and metabolites ($k_{ep}$ and $k_{em}$) were equal (0.040 ± 0.008 and 0.040 ± 0.005 h$^{-1}$). The FU biotransformation rate ($k_m = 0.048 \pm 0.004$ h$^{-1}$) was relatively slow compared to PCP and MP and is likely due to the difference in the metabolic systems that are responsible for the biotransformation among the different compounds. The BCF calculated from the kinetics for FU was 2095. The biological half-life of

**Table 1.** Toxicokinetic model parameters (± SD) for pentachlorophenol, methyl parathion, fluoranthene, and hexachlorobiphenyl by *Hyalella azteca* in artificial fresh water at pH 7.8 and some chemical characteristics of the studied compound

| Parameter | PCP | Methyl parathion | Fluoranthene | HCBP |
|---|---|---|---|---|
| $k_u$ (ml $g^{-1}$ $h^{-1}$) | 25.7 ± 2.9 | 11.5 ± 1.1 | 184.4 ± 9.3 | 251.7 ± 9.0 |
| $k_{ep}$ ($h^{-1}$) | 0.061 ± 0.034 | 0.403 ± 0.070 | 0.040 ± 0.008 | 0.004 ± 0.001 |
| $k_{em}$ ($h^{-1}$) | 0.076 ± 0.012 | 0.021 ± 0.001 | 0.040 ± 0.005 | ND |
| $k_m$ ($h^{-1}$) | 0.133 ± 0.017 | 0.539 ± 0.095 | 0.048 ± 0.004 | ND |
| $k_e$ ($h^{-1}$)[1] | 0.068 ± 0.012 | 0.015 ± 0.002 | 0.026 ± 0.002 | 0.004 ± 0.001 |
| Kinetic BCF[2] | 132 | 12 | 2095 | 69,917 |
| Regression BCF[3] | 479[4] 36[5] | 3.3 | 2358 | 45,362 |
| $t_{1/2}$ (h) parent[6] | 3.6 | 0.7 | 7.9 | 193 |
| $t_{1/2}$ (h) metabolite[7] | 9.1 | 33.1 | 17.2 | ND |
| COD[8] | 0.76 | 0.90 | 0.83 | 0.83 |
| Log $K_{ow}$ | 4.45[4], 3.2[5] | 2.04[9] | 5.22[10] | 6.65[9] |
| Water solubility (mg/L) | 14 (20°C)[9] | 55–60 (25°C)[9] | 0.265 (25°C)[9] | 0.0088 (25°C)[11] |

ND = not determined.
[1] $k_e$ = total compound elimination rate constant ($h^{-1}$).
[2] BCF = $(k_u)\cdot(k_{ep} + k_m)^{-1}$ Equation (11).
[3] Calculated BCF taken from the regression Log BCF = 0.898 Log $K_{ow}$ − 1.315 (Hawker and Connell 1986).
[4] Using log $K_{ow}$ from Westhall (1985).
[5] Log $K_{ow}$ value for pH 7.8 from Kaiser and Valdmanis (1982).
[6] $t_{1/2} = (0.693) \cdot (k_{ep} + k_m)^{-1}$.
[7] $t_{1/2} = 0.693 \cdot k_{em}^{-1}$.
[8] Coefficient of Determination.
[9] Verschueren (1983).
[10] Mackay *et al.* (1991).
[11] Wallnofer *et al.* (1973).

the parent compound ($t_{1/2p}$) was 7.9 h, and half-life of metabolites ($t_{1/2m}$) was 17.2 h (Table 1).

### *Hexachlorobiphenyl*

The uptake clearance ($k_u$) of HCBP (251.7 ± 9.0 ml $g^{-1}$ $h^{-1}$) was the most rapid of all the compounds (Fig. 2), while the elimination rate ($k_{ep}$ = 0.004 ± 0.001 $h^{-1}$) was over two orders of magnitude slower reflecting the large log $k_{ow}$ (6.65) value for this compound. HCBP was not analyzed for biotransformation, since it is generally recognized that many species, including fish, show little ability to biotransform highly chlorinated PCB congeners (Stein *et al.* 1987). Thus, the kinetics for HCBP was simplified. The measured BCF was 69,917 at the end of the exposure, and the calculated BCF from the kinetics was 45,362. The biological half-life of the compound was 193 h (Table 1).

## Discussion

In this study, uptake clearances ($k_u$) generally increased with increasing log $K_{ow}$ of the parent compound, while the elimination of the parent compound ($k_{ep}$) was inversely proportional to log $K_{ow}$ indicating that the more hydrophilic compounds were poorly accumulated and more readily eliminated. This relationship between the hydrophobicity of a compound (log $K_{ow}$) and its relative uptake and elimination rates is well documented (Lohner and Collins 1987; Landrum 1989; Lydy *et al.* 1992). On the other hand, the rates of biotransformation also appeared to be inversely related to the log $K_{ow}$ of the compound. This likely occurs because the more hydrophilic compounds tend to have more reactive functional groups in their structures. While specific metabolites were not determined in this study, it is expected that the relationship between the rate of biotransformation and the hydrophobicity of the compound is complicated by the route and type of metabolite that is formed for each of the compounds. For instance, the biotransformation of PCP is expected to proceed via phase II conjugation only, while the other compounds must first undergo some form of phase I biotransformation (Stehly and Hayton 1988).

The kinetics determined in this study are more complex than are generally modeled for aquatic invertebrates. As a result, the fate of the parent compound and the metabolites are better defined. However, the increased detail makes comparison with other work difficult. This particularly affects comparison of the elimination rates in this work with the overall elimination rates often presented. When the elimination rate is modeled by a single constant for total radioactivity, it is not known what compound formation actually is the rate-controlling step, elimination of parent compound or metabolite. For instance, if estimates of $k_e$ for total elimination of MP (0.015 $h^{-1}$) were to be evaluated against the detailed kinetics, it is clear from the data (Table 1) that the overall elimination of radioactivity is primarily limited by the rate of elimination of the metabolite (0.021 $h^{-1}$). The balance of the total elimination is nearly equal between parent and metabolite for FU and dominated by the rate of elimination of parent for HCBP. When comparing to other studies where a single total elimination constant is available, it may be necessary to compare to the rate constant for parent, metabolite, or both depending on the compound and the extent of biotransformation.

In the case of PCP, the compound is mainly ionized at pH

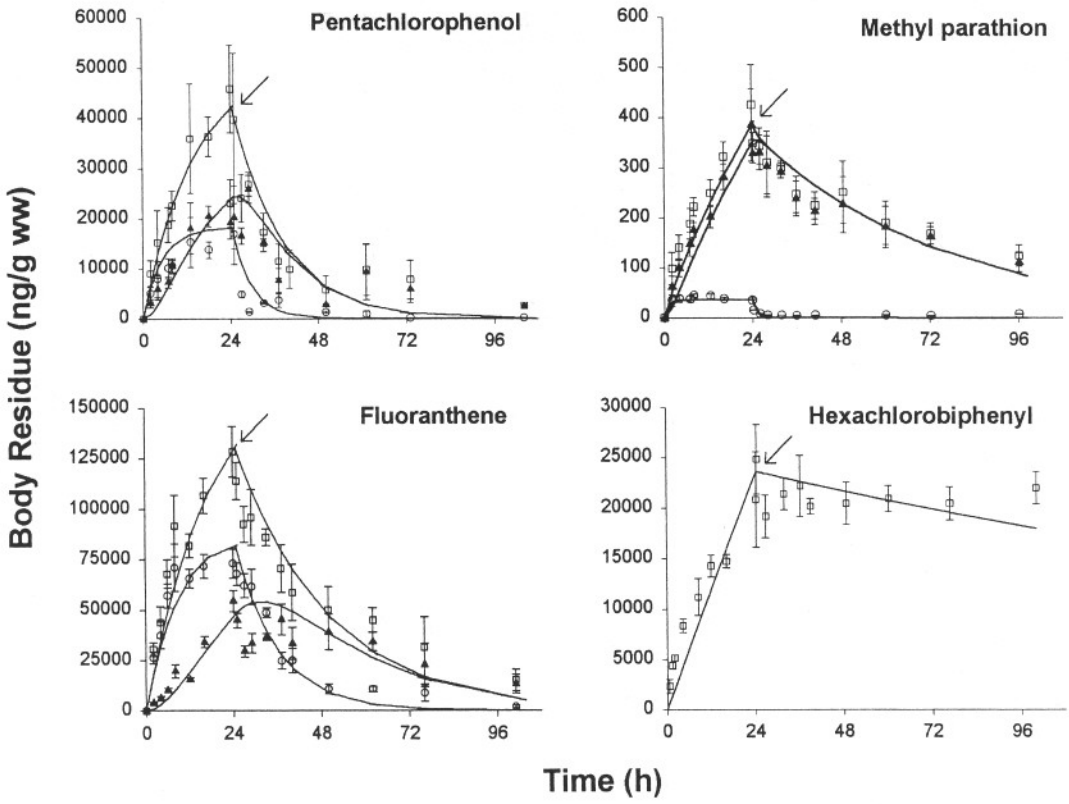


**Fig. 2.** Toxicokinetics of pentachlorophenol, methyl parathion, fluoranthene, and hexachlorobiphenyl by *Hyalella azteca* in moderately hard water: □ = total compound, ▲ = polar metabolites, and ○ = parent compound; the lines represent the predicted points from the model. The depuration phase started at 24 h and is marked with an arrow

7.8–7.9, thus a high accumulation rate is not expected. This is because the rate of uptake of the ionized form is expected to be much slower than that for the non-ionized form. The overall $k_u$ ($25.7 \pm 2.9$ ml $g^{-1}$ $h^{-1}$) for PCP was slow compared to uptake rates in studies with *H. azetca* with other compounds. For example, Landrum and Scavia (1983) found a much faster uptake clearance ($218 \pm 27$ ml $g^{-1}$ $h^{-1}$) for *H. azetca* exposed to anthracene. This finding was initially surprising, since anthracene has a similar log $K_{ow}$ value (4.54) to PCP (4.45). However, if the effective log $K_{ow}$ value for PCP at pH 7.8 is used (3.2, Kaiser and Valsmanis 1982), then the slower $k_u$ value found for PCP is more in line with expectations. On the other hand, the uptake clearances for PCP found in the current study compare fairly well with those observed for other organisms: $3.1 \pm 0.9$ ml $g^{-1}$ $h^{-1}$ for *Diporeia* spp. at pH 8 and 4°C (Landrum and Dupuis 1990), 39.5 ml $g^{-1}$ $h^{-1}$ for goldfish at pH 7 and 20°C (Stehly and Hayton 1990), 55.5 ml $g^{-1}$ $h^{-1}$ for *Chironomus tentans* at pH 7 and 20°C (Lydy *et al.* 1994) and 42.3 ml $g^{-1}$ $h^{-1}$ for *Dreissena polymorpha* at pH 8.5 and 25°C (Fisher *et al.* 1999). The small differences that were noted among these species can be attributed to functional issues such as the high filtering rates of bivalves, physical characteristics such as organism size and lipid content that can affect measured uptake rates or differences in exposure conditions such as pH and temperature (Landrum 1988).

PCP was metabolized by *H. azetca* at a fairly fast rate ($0.133 \pm 0.017 h^{-1}$). In general, phenols are metabolized directly by phase II conjugation reactions, thus accounting for the relatively rapid metabolite formation. This rate is considerably faster than that for FU ($0.040 \pm 0.005$ $h^{-1}$), which must be transformed by oxidative metabolism with systems such as cytrochrome P450. Conjugation reactions for PCP generally involve sulfate or glucuronide conjugates (Stehly and Hayton 1988). In this study, polar PCP metabolites were eliminated ($0.076 \pm 0.012$ $h^{-1}$) at the same rate as the parent compound ($0.061 \pm 0.034$ $h^{-1}$). The relatively slow elimination of the conjugated metabolites and parent compound likely results from the difficulty of the ionic form in passing through the respiratory membrane, the likely site of elimination of these compounds (Landrum and Crosby 1981).

Methyl parathion is not accumulated as rapidly ($11.5 \pm 1.1$ ml $g^{-1}$ $h^{-1}$) as the other compounds tested in this study and that is because it has the smallest log $K_{ow}$ value of the group (2.04, Verschueren 1983). A literature review found no toxicokinetic studies performed with aquatic invertebrates for MP; however, de Bruijn *et al.* (1991) measured toxicokinetic parameters for MP in fish (*Poecilia reticulata*). The uptake clearance was $0.11 \pm 0.04$ ml $g^{-1}$ $h^{-1}$ and the slower uptake for *P. reticulata* compared to *H. azteca* was likely due to the larger size of the fish compared to the amphipod.

Metabolite formation of MP in *H. azetca* was rapid ($k_m = 0.539 \pm 0.095$ $h^{-1}$), which likely reflects the number of routes available for degradation including metabolism by esterases, hydrolases, oxidases, and gluathione s-transferases (Lydy *et al.* 1999; Chambers and Levi 1992). In a study by Gunning *et al.* (1994), MP also was rapidly metabolized and excreted by the

terrestrial insect larvae *Helicoverpa armigera* and *H. punctivera* with p-nitrophenol identified as the dominant metabolite. Polar metabolites were eliminated slowly from *H. azetca* ($0.021 \pm 0.001\ h^{-1}$) compared to the elimination of parent MP ($0.403 \pm 0.070\ h^{-1}$) and compared to the elimination of the PCP and FU metabolites. This may result from the formation of bound metabolites associated with the receptor. One of the metabolites, methyl paraoxon, binds almost irreversibly to the cholinesterase receptor (Kallander *et al.* 1997), which partly explains the slow elimination of MP metabolites ($t_{1/2m}$ = 33.1 h). The significantly shorter biological half-life of the parent compound ($t_{1/2p}$ = 0.7 h) takes into account both the actual elimination of the parent compound and its biotransformation into metabolites.

The uptake clearance ($k_u$) for FU ($184.4 \pm 9.3\ ml\ g^{-1}\ h^{-1}$) by *H. azteca* was similar to that measured by Kane Driscoll *et al.* (1997b) for the same species. Their $k_u$ values ranged from 284 to 439 ml $g^{-1}$ $h^{-1}$. It also was similar to the uptake rate found for anthracene by *H. azteca* $255 \pm 76\ ml\ g^{-1}\ h^{-1}$ (Landrum and Scavia 1983). This was likely the case, since the log $K_{ow}$ values for anthracene and FU are fairly similar (4.54 versus 5.22). The parent compound elimination rate constant ($k_{ep}$) for FU was $0.040 \pm 0.008\ h^{-1}$, and was rather slow compared to those measured by Kane Driscoll *et al.* (1997b). They found elimination rates that ranged from 0.128 to 0.188 $h^{-1}$. The elimination of the FU metabolites ($0.040 \pm 0.005\ h^{-1}$) was as slow as the elimination of parent compound (Table 1). The slow elimination of the metabolites was similar to that found for the other compounds. This also reflects the possible influence of resistance of membranes to the passage of polar compounds reducing the rate of loss. The metabolite formation rate for FU was slow ($0.048 \pm 0.004\ h^{-1}$) compared to the other studied compounds. Biotransformation requires that oxidative metabolism, such as through a P450 enzyme system, insert a functional group into fluoranthene. This process is less likely available than simple conjugation or ester hydrolysis, because of either lower enzyme titers or greater energetic requirements or both. The rate of FU metabolism by *H. azteca* was greater in this study than found previously (Kane Driscoll *et al.* 1997b). After 24 h, 57% of the total body burden was present as parent FU in the present study compared to 83% found in the Kane Driscoll *et al.* (1997b) study.

The uptake clearance ($k_u$) for HCBP ($251.7 \pm 9.0\ ml\ g^{-1}\ h^{-1}$) was the largest among the compounds tested and reflects HCBP's larger log $K_{ow}$ (6.65). The uptake clearance rate was larger than those measured by Evans and Landrum (1989) for *Diporeia* spp. and *Mysis relicta* 53.5 ml $g^{-1}$ $h^{-1}$ and 57.5 ml $g^{-1}$ $h^{-1}$, respectively. Conversely, the $k_u$ for *Dreissena polymorpha* ($1102 \pm 356\ ml\ g^{-1}\ h^{-1}$) was larger than that for *H. azteca* likely due to the high filtering rate of the mussels (Gossiaux *et al.* 1996). However, the $k_u$ value for *H. azetca* was within the range found for *Diporeia* spp. 66–392 ml $g^{-1}$ $h^{-1}$ over a range of temperatures of 4–12°C and mass of organisms from 1.8–14 mg wet weight (Landrum *et al.* 2001). The parent compound elimination rate constant ($k_{ep}$) by *H. azteca* for HCBP was $0.004 \pm 0.001\ h^{-1}$ in the present study, which is faster than those measured for *Diporeia* spp. (0.0008 $h^{-1}$, Evans and Landrum 1989; 0.0006 $h^{-1}$, Landrum *et al.* 2001). On the other hand, *D. polymorpha* had a similar elimination rate constant value ($0.004 \pm 0.0001\ h^{-1}$, Gossiaux *et al.* 1996).

Calculated bioconcentration factors (BCFs) from regression with the octanol/water partition coefficient (Hawker and Connell 1986) were in general agreement with those estimated from the kinetics for the parent compound, but did not reflect the total bioaccumulated residue (Table 1). This was mainly because the biotransformation observed in *H. azteca* could not be accounted for in the kinetic equations. The BCF estimated from the kinetics for PCP was 132 and in good agreement with the BCF for *Mysis relicta* (128) at pH 8 (Landrum and Dupuis 1990). At lower pH's with reduced ionization, the BCF values for PCP in the literature were higher. For example, the BCF for *Anodonta anatina* varied from 145 to 342 at pH 6.5 (Mäkela and Oikari 1990) and for *Chironomus riparius* the BCF was 458 at pH 6 (Lydy *et al.* 1994). The extent of ionization coupled with species differences in lipid content and filtration rates most likely influenced these BCF estimates. The BCF estimated from the kinetics is lower than that calculated from the log $K_{ow}$ relationship (479) if the log $K_{ow}$ of the non-ionized PCP is used, this difference can be explained both by biotransformation and ionization of PCP. Accounting for the effect of ionization (Kaiser and Valmanis 1982) on log $K_{ow}$, the BCF estimated from the regression is lower (36) than that estimated from the kinetics. This is likely the result of either not accurately accounting for the impact of pH on log $K_{ow}$ or more likely the limit of using a regression equation for non-polar hydrophobic compounds on a polar ionized compound.

Methyl parathion has a low log $K_{ow}$ of 2.04 (Verscheuren 1983) and the combination of this along with the very high biotransformation rate accounts for the low BCF estimated from the kinetics (12). Similar BCFs have been reported for the guppy (9.6) (de Bruijn and Hermens 1991) and for the decopod, *Cambarellus montezumae* (9), the mollusk, Planorbidae (17) and the macrophyte, *Lemna* sp. (7) in a freshwater community in Mexico (De la Vega Salazar *et al.* 1997). The estimated BCF from the reported log $K_{ow}$ (3.3) is lower than that estimated from the kinetics (12). Thus, the empirical regression equation appears to be performing poorly for MP not accounting for the high biotransformation rate for this compound.

The BCFs for both FU and HCBP estimated from the kinetics are close to those calculated from the log $K_{ow}$ regression, although the estimated value from the regression equation for HCBP was a little low. The BCF estimated from the kinetics for HCBP could be artificially elevated due to the error associated with the estimate of $k_{ep}$ ($0.004 \pm 0.001$), which has a relatively large standard deviation (25% error) or because the log $K_{ow}$ and BCF relationship breaks down for compounds with log $K_{ow}$ values greater than 6 (HCBP log $K_{ow}$ = 6.65).

Recent efforts to employ body residue as a dose metric has focused on parent compound concentrations (McCarty and Mackay 1993). However, this work has shown that there is a substantial buildup of metabolites in *H. azteca* exposed to several compounds of differing chemical classes. Currently, with the exception of compounds like MP where the oxon metabolite is recognized as the proximal toxicant, the impact of metabolites on toxicity are generally not known or studied. If the use of accumulated body residues is to be effective as a dose metric, then the impact of metabolite accumulation will need to be determined.

This was among the first studies to examine the kinetics of biotransformation of xenobiotics in aquatic invertebrates with multiple classes of compounds. It is clear that there is a buildup

of metabolites that may well contribute to the total body residue of the contaminants and contribute to the toxic response either through some specific mode of action or through a non-polar mechanism of action. However, the extent of metabolite contribution to toxicity is not currently known. Thus, if metabolites contribute to the toxicity of contaminants, new bioaccumulation models will need to be developed to predict total effective residue in these organisms to improve hazard interpretation.

*Acknowledgments.* This study was financed by a US Environmental Protection Agency Experimental Program to Stimulate Competitive Research (EPAEPSCoR), grant R827589-01-0. The authors also wish to thank Merja Lyytikäinen, Nate Davis, and Troy Anderson for their help with the laboratory work. GLERL contribution no. 1259.

## References

ASTM (2000) Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates. In: *Annual book of ASTM standards, water and environmental technology. volume 11.05* (E1706-95b). American Society for Testing and Materials, Philadelphia, pp 1129–1211

Blockwell SJ, Maund SJ, Pascoe D (1999) Effects of the organochlorine insecticide lindane ($\gamma$-$C_6$ $H_6$ $Cl_6$) on the population responses of the freshwater amphipod *Hyalella azteca*. Environ Toxicol Chem 18:1264–1269

Chambers HW (1992) Organophosphorus compounds: an overview. In: Chambers JE, Levi PE (eds) Organophosphates: chemistry, fate and effects. Academic Press, San Diego, pp 1–17

de Bruijn J, Hermens J (1991) Uptake and elimination kinetics of organophosphorous pesticides in the guppy (*Poecilia reticulata*): correlations with the octanol/water partition coefficient. Environ Toxicol Chem 10:791–804

De la Vega Salazar M, Martinez Tabche L, Macias Garcia C (1997) Bioaccumulation of methyl parathion and its toxicity in several species of the freshwater community in Ignacio Ramirez Dam in Mexico. Ecotoxicol Environ Safety 38:53–62

Duan Y, Guttman SI, Oris JT, Huang X, Burton GA (2000) Genotype and toxicity relationships among *Hyalella azteca:* II. Acute exposure to fluoranthene-contaminated sediment. Environ Toxicol Chem 19:1422–1426

Evans MS, Landrum PF (1989) Toxicokinetics of DDE, benzo-(a)pyrene, and 2,4,5,2′,4′,5′-hexachlorobiphenyl in *Pontoporeia hoyi* and *Mysis relicta*. J Great Lakes Res 15:589–600

Fisher SW, Hwang H, Atanasoff M, Landrum PF (1999) Critical body residues for pentachlorophenol in zebra mussels (*Dreissena polymorpha*) under varying conditions of temperature and pH. Ecotoxicol Environ Safety 43:1–14

Gossiaux DC, Landrum PF, Fisher SW (1996) Effect of temperature on the accumulation kinetics of PAHs and PCBs in the zebra mussel, *Dreissena polymorpha*. J Great Lakes Res 22:379–388

Gunning RV, Ferris IG, Easton CS (1994) Toxicity, penetration, tissue distribution, and metabolism of methyl parathion in *Helicoverpa armigera* and *H. punctigera* (Lepidoptera: Noctuidae). J Econ Entomol 87:1180–1184

Hawker DW, Connell DW (1986) Bioconcentration of lipophilic compounds by some aquatic organisms. Ecotoxicol Environ Safety 11:184–197

Ingersoll CG, Ivey CD, Brunson EL, Hardesty DK, Kemble NE (2000) Evaluation of toxicity: whole-sediment versus overlaying-water exposures with amphipod *Hyalella azteca*. Environ Toxicol Chem 19:2906–2910

Kallander DB, Fisher SW, Lydy MJ (1997) Recovery following pulsed exposures to organophosphorus and carbamate insecticides in the midge, *Chironomus riparius*. Arch Environ Contam Toxicol 33: 29–33

Kaiser KLE, Valdmanis I (1982) Apparent octanol/water partition coefficients of pentachlorophenol as a function of pH. Can J Chem 60:2104–2106

Kane Driscoll S, Harkey GA, Landrum PF (1997a). Accumulation and toxicokinetics of fluoranthene in sediment bioassays with freshwater amphipods. Environ Toxicol Chem 16:742–753

Kane Driscoll S, Landrum PF, Tigue E (1997b) Accumulation and toxicokinetics of fluoranthene in water-only exposures with freshwater amphipods. Environ Toxicol Chem 16:754–761

Landrum PF (1988) Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi:* role of physiological and environmental variables. Aquat Toxicol 12:245–271

Landrum PF (1989) Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi*. Environ Sci Tech 23:588–595

Landrum PF, Crosby DG (1981) Comparison of the disposition of several nitrogen-containing compounds in the sea urchin and other marine invertebrates. Xenobiotica 11:351–361

Landrum PF, Scavia D (1983) Influence of sediment on anthracene uptake, depuration, and biotransformation by the amphipod *Hyalella azteca*. Can J Fish Aquat Sci 40:298–305

Landrum PF, Dupuis WS (1990) Toxicity and toxicokinetics of pentachlorophenol and carbaryl to *Pontoporeia hoyi* and *Mysis relicta*. In: Landis WG, van der Schalie WH (eds) *Aquatic toxicology and risk assessment: thirteenth volume, ASTM STP 1096*. American Society for Testing and Materials, Philadelphia, pp 278–289

Landrum PF, Lee H, Lydy MJ (1992) Toxicokinetics in aquatic systems: model comparison and use in hazard assessment. Environ Toxicol Chem 11:1709–1725

Landrum PF, Tigue EA, Kane Driscoll S, Gossiaux DC, Van Hoof PL, Gedeon ML, Adler M (2001) Bioaccumulation of PCB congeners by *Diporeia* spp.: kinetics and factors affecting bioavailability. J Great Lakes Res 27:117–133

Lofuto GR, Landrum PF, Gedeon ML, Tigue EA, Herche LR (2000) Comparative toxicity and toxicokinetics of DDT and its major metabolites in freshwater amphipods. Environ Toxicol Chem 19: 368–379

Lohner TW, Collins WJ (1987) Determination of uptake rate constants for six organochlorines in midge larvae. Environ Toxicol Chem 6:137–146

Lydy MJ, Oris JT, Baumann PC, Fisher SW (1992) Effects of sediment organic carbon content on the elimination rates of neutral lipophilic compounds in the midge *Chironomus riparius*. Environ Toxicol Chem 11:347–356

Lydy MJ, Hayton WL, Staubus AE, Fisher SW (1994) Bioconcentration of 5,5′,6-trichlorophenyl and pentachlorophenol in the midge, *Chironomus riparius*, as measured by pharmacokinetic model. Arch Environ Contam Toxicol 26:251–256

Lydy MJ, Belden JB, Ternes MA (1999) Effects of temperature on the toxicity of m-parathion, chlorpyrifos, and pentachlorobenzene to *Chironomus tentans*. Arch Environ Contam Toxicol 37:542–547

Lydy MJ, Lasater JL, Landrum PF (2000) Toxicokinetics of DDE and 2-chlorobiphenyl in *Chironomus tentans*. Arch Environ Contam Toxicol 38:163–168

Mackay D, Shiu WY, Ma KC (1991) Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals, polynuclear aromatic hydrocarbons, polychlorinated dioxins, and dibenzofurans. Lewis Publishers, Chelsea, MI

Mäkela P, Oikari AOJ (1990) Uptake and body distribution of chlorinated phenolics in the freshwater mussel, *Anodonta anatine*. L Ecotoxicol Environ Safety 20:354–362

McCarty LS, Mackay D (1993) Enhancing ecotoxicological modeling and assessment. Environ Sci Technol 27:1719–1728

McNulty EW, Dwyer FJ, Ellersieck MR, Greer EI, Ingersoll CG, Rabeni CF (1999) Evaluation of ability of reference toxicity tests to identify stress in laboratory populations of the amphipod *Hyalella azteca*. Environ Toxicol Chem 18:544–548

Schuler LJ, Heagler MG, Lydy MJ (2002) Bioavailability within single versus multiple species systems. Arch Environ Contam Toxicol 42:199–204

Steevens JA, Benson WH (2001) Toxicokinetic interactions and survival of *Hyalella azteca* exposed to binary mixtures of chlorpyrifos, dieldrin and methyl mercury. Aquat Toxicol 51:377–388

Stehly GR, Hayton WL (1988) Detection of pentachlorophenol and its glucuronide and sulfate conjugates in fish bile and exposure water. J Environ Sci Health B23:355–366

Stehly GR, Hayton WL (1990) Effect of pH on the accumulation kinetics of pentachlorophenol in goldfish. Arch Environ Contam Toxicol 19:464–470

Stein JE, Hom T, Casillas E, Friedman A, Varanasi U (1987) Simultaneous exposure of English sole (*Parophrys vetulus*) to sediment-associated xenobiotics: part 2—chronic exposure to an urban estuarine sediment with added $^{3}$H-benzo[a]pyrene and $^{14}$C-polychlorinated biphenyls. Mar Environ Res 22:123–149

USEPA (2000) Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, Second ed, EPA 600/R-99/064. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN

Verschueren K (1983) Handbook of environmental data on organic chemicals, second ed. Van Nostrand Reinhold, New York

Wallnofer PR, Koniger M, Hutzinger O (1973) The solubilities of twenty-one chlorobiphenyls' in water. Analab Res Notes 13: 14–19

Westhall JC (1985) Influence of pH and ionic strength on the aqueous-nonaqueous distribution of chlorinated phenols. Environ Sci Technol 19:193–198